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Transposable Elements in Rice Plants

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Abstract

Two types of transposable elements in rice plants were characterized after isolation. One is closely related to Ac/Ds elements of maize and thus named RAc. The RAc elements are present in wild rice species as well as in cultivated rice species. The distribution of RAc elements in the genomes is different even among the closely related cultivars, suggesting that the RAc elements be active. The other is a retrotransposon, the structure of which resembles the integrated forms of retroviruses. Two general methods have been developed to isolate retrotransposon of plants. By using these methods, at least 12 families of retrotransposons of rice (Tos1-Tos12) were isolated. One retrotransposon, Tos3-1, was subjected to detailed investigation. Tos3-1 is 5.2 kb long and has structures common to retrotransposons of yeast and *Drosophila*. Southern blotting analysis showed that retrotransposons were present in wild rice species as well as in cultivated rice species, but not in maize and tobacco. The copy number and genomic location of some families varied among the cultivars tasted, suggesting that these elements be active. Total copy number of the retrotransposons was estimated to be approximately 1,000 in the rice genome. These results indicate that the retrotransposons are major transposons in rice as the case in *Drosophila*. The transposable elements described in this paper are the first ones found in rice.

Discipline: Biotechnology
Additional key words: Ac, evolution, retrotransposons, reverse transcriptase

Introduction

Transposable elements are mobile genetic elements capable of moving from one site to another in the genome. Nearly 40 years ago, McClintock first described transposable elements in maize. Since then, many transposable elements have been found in different organisms including bacteria, yeast, *Drosophila* and human as well[1]. Molecular and genetic studies on these elements have provided valuable insights into the regulatory mechanisms of gene expression and the dynamics of the eukaryotic genome in addition to the mechanism of transposition itself. In recent years, transposable elements have drawn attention as a tool to identify and clone genes of interest[6]. This cloning procedure is called transposon tagging or gene tagging. Using transposon tagging, it is possible to clone genes which cannot be cloned with ordinary cloning methods. The relevant examples include genes involved in development, growth, morphology and agronomically important traits. The usefulness of this procedure in gene cloning has been well recognized in maize and snap dragon (*Antirrhinum majus*). However, transposon-tagging system is not available in other plants, because active transposable elements have not been isolated. Strenuous efforts are being made to develop the tagging system in these plants by isolating new transposable elements or by introducing the transposable elements of maize and snap dragon into these plants[6].

Transposable elements are classified into two types. The first type comprises elements such as the well-

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characterized Ac/Ds elements of maize[10] and Tam elements of snap dragon[7]. The second type comprises the retrotransposons which have been well characterized in yeast[4] and *Drosophila*[2], structurally resembling the integrated forms of retroviruses. Those two types are different from each other functionally as well as structurally. The former one excises from one site in the genome and reintegrates into new sites in the genome. Thus, this type of transposable elements induces unstable mutations due to frequent excision. Retrotransposons, in general, do not induce unstable mutations because they undergo the replicative transposition through RNA as an intermediate. So, it is not easy to identify the retrotransposons with a genetic analysis. Most of the retrotransposons of plants were therefore identified only recently through molecular approaches[5,12,25,31,37].

In maize, approximately 10 transposable elements were found with a genetic analysis; three of which, i.e. Ac, Spm, and Mu elements, were studied at a molecular level[10] and used for transposon tagging[6]. No such elements have so far been reported in rice plants, although maize and rice are related, both belonging to the same family *Gramineae*. This paper attempts to review some results obtained in the studies on cloning and characterization of transposable elements of rice, and discuss their possible uses for practical purposes.

Cloning and structural analysis of Ac/Ds-like transposable elements

Ac/Ds elements are the first transposable elements found in maize. The Ac elements are autonomous, whereas the Ds elements are non-autonomous derivatives of the Ac elements. An Ac-like sequence of pearl millet (*Pennisetum glaucum*) has been cloned recently[19]. A structural analysis on Tam3 element of snap dragon revealed its homologies to the Ac element[13]. These results suggest that Ac-like elements be widely distributed in plants. The presence of such elements in rice genome was examined by low stringent hybridization using the Ac clone as a probe. A genomic library from a wild rice, *Oryza australiensis*, was constructed by cloning *Sau3A* partially digested total genomic DNA into the *Bam*HI site of EMBL3 vector. This library was screened under the low stringent hybridization condition using the entire sequence of the maize Ac element, i.e. 4.6 kb[22]. Two positive clones were obtained[14] and Ac-related sequences were mapped on the cloned

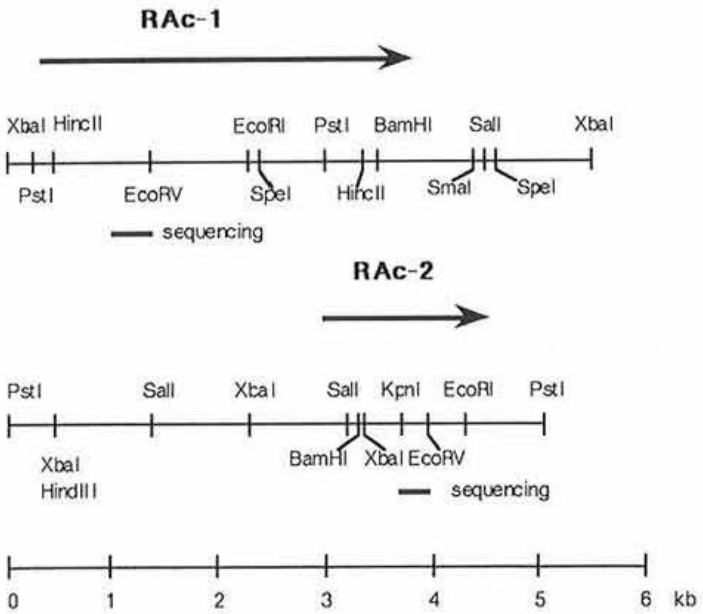

Fig. 1. Restriction maps of the genomic clones containing RAc-1 and RAc-2

sequences based on the hybridization with the ^{32}P-labelled Ac probe (Fig. 1). These sequences homologous to the Ac element were named RAc-1 and RAc-2, respectively. The sizes of RAc-1 and RAc-2 were estimated to be approximately 3.5 and 1.5 kb, respectively. One region of both elements was sequenced and their sequences were compared with the sequence of the Ac element. The RAcs shared 93% nucleotide identity, indicating that these elements belonged to the same family. The RAcs showed 66 and 77% homology to the Ac element at nucleotide and amino acid levels, respectively.

Distribution and activity of Ac/Ds-like elements in the genome

The low stringent hybridization of the rice genomes with the RAc probe showed that Ac-like elements were widely distributed in the genus *Oryza* including wild and cultivated rice species. One example of the hybridization experiments was shown in Plate 1. The genomic DNAs of five cultivars each from indica and japonica subspecies of *O. sativa* were digested with *Hin*dIII and hybridized with ^{32}P-labelled RAc-1 probe. About 15 to 20 hybridization bands were detected in each genome and some of them were polymorphic among the cultivars tested. The band patterns were more variable between those two subspecies. The same results were obtained, using another restriction enzyme *Eco*RI. Three factors induce the band polymorphism. They are point mutations and methylation of DNA, and transposition. Involvement of the point mutations are unlikely, because the polymorphism is observed even among the closely related cultivars, such as Koshihikari, Nipponbare, Norin 10 and Norin 29. Methylation occurs only in the C residue of the CG or CNG sequence in plants[30]. So, *Hin*dIII site has no methylation site, denying the involvement of DNA methylation. The polymorphism mentioned above can be explained only by transposition of the RAc elements. In the maize genome, approximately 10 to 20 copies of the Ac-related sequences were found[9]. However, only a few copies of them are active in transposition. In rice, most of the Ac-like sequences do not show the polymorphism among the cultivars and the polymorphism is restricted to some bands. These bands should correspond to the RAc capable of transposing. The frequency and the regulation of transposition of these elements are now being worked out.

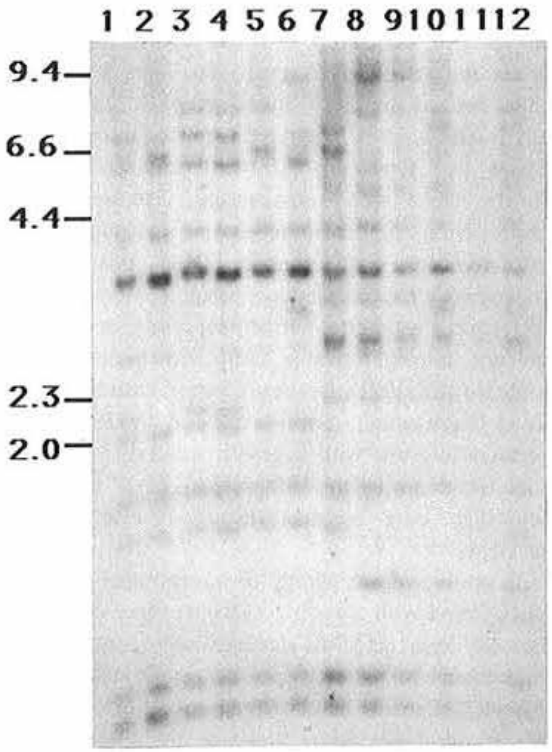

Plate 1. Distribution of RAc-related sequences in japonica and indica rice
The genomic DNAs were digested with *Hin*dIII and probed with the labelled *Xba*I–*Bam*HI fragment of RAc-1 (see Fig. 1).
Lanes 1–6: japonica cultivars Aikoku, Norin 10, Norin 29, Koshihikari, Fujisaka 5 and Nipponbare, respectively.
Lanes 7–12: indica cultivars Dee-geo-woo-gen, Taichung Native 1, Tsai-yuan-chung, IR 8, Culture 340, and Peku, respectively.

Cloning of retrotransposons

Retrotransposons are transposable elements characterized by the presence of long terminal repeats (LTRs) and transpose via an RNA intermediate using reverse transcriptase encoded by a *pol* gene, as clearly demonstrated in yeast[3]. Reverse transcription is primed by a transfer RNA (tRNA), the 3' end of which hybridizes to the primer binding site (PBS) adjacent to the 5' LTR. Several tRNA species have been found to be made available, but a given species

is characteristic for each retrotransposon in animals[2]. However, all of the plant retrotransposons whose sequences have been determined have the identical PBS, a sequence of which is complementary to the initiator methionine tRNA[5,11,18,26,31,32]. These results suggest that the initiator tRNA be preferentially used as a primer in plants, thus providing a general means to clone plant retrotransposons. The genomic DNA library of japonica rice was screened for retrotransposons, using an oligonucleotide complementary to the 14 bases at the 3' end of plant initiator tRNA[27]. Although the oligonucleotide is also homologous to the tRNA genes, the hybridization to tRNA genes can be eliminated under selective hybridization-washing conditions because they lack the terminal CCA sequence, which is added post-transcriptionally. Assuming the genome size of *O. sativa* to be 6.7×10^5 kb[17] and the average genomic DNA insert size to be 15 kb, one genome equivalent to 4.5×10^4 plaques was screened. Approximately 1,000 positive plaques were detected, from which three positive plaques were selected at random and purified further. The DNA fragments hybridizing to the oligonucleotide probe were subcloned and their nucleotide sequences were determined[16]. The sequence analysis showed that three clones carried different retrotransposons: these were named Tos1-1, Tos2-1, and Tos3-1 (referring to transposon of *O. sativa*). The structure of Tos3-1 was examined in detail (Fig. 2). The total size of this element was approximately 5.2 kb. The nucleotide sequence of LTRs and their flanking sequences were determined (Fig. 2 B). The Tos3-1 sequence was flanked by direct repeats of five base pairs. It was shown that this sequence was the consequence of a duplication of the target sequence by analyzing the corresponding unoccupied sequence of the indica rice (see the next section). The length of each of the LTRs was 115 bp and they shared 95% nucleotide identity. A polypurine tract (PPT) was located

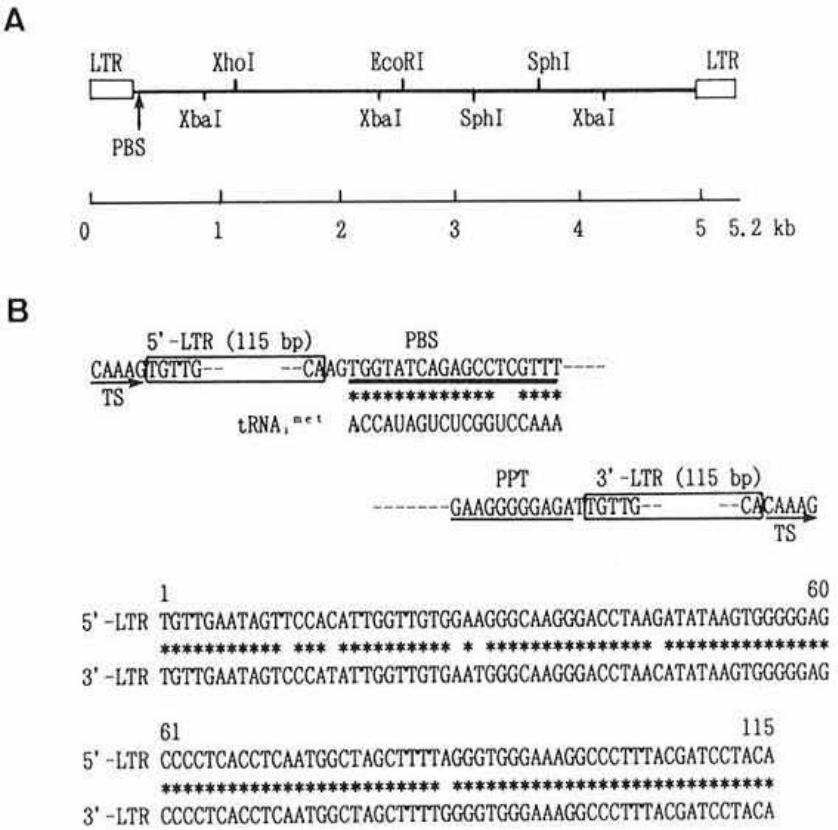

Fig. 2. Structural features of Tos3-1

upstream of 3'-LTR and could serve to prime plus strand DNA synthesis[2].

Certain amino acid sequences in the reverse transcriptase domain are well conserved among the retrotransposons[8]. The plant retrotransposons Ta1-3 from *Arabidopsis thaliana*[32] and Tnt1 from *Nicotiana tabacum*[11] share those sequences which are quite similar to those of the copia element of *Drosophila melanogaster*[21]. Two oligonucleotide primers corresponding to two conserved motifs (QMDVKT and YVDDM) were made and utilized to amplify the suspected reverse transcriptase domain of the rice retrotransposons by the polymerase chain reaction (PCR)[16]. The sequences amplified from japonica rice genomic DNA were cloned into M13 vector and 35 clones were sequenced. These sequences were classified into 7 families on the basis of amino acid sequence homology and showed homology to the copia element (Fig. 3). Sequences at the amino and carboxyl ends corresponding to the oligonucleotide primers used are not included in Fig. 3, because they may not represent the true genomic sequences. The retrotransposons carrying these sequences are named Tos4, 5, 6, 7, 8, 9, 10, 11, and 12, respectively. By using the cloned sequences of Tos4 and Tos5 as probes, the genomic library was screened. About half of the positive clones detected by each probe had sequences homologous to the PBS oligonucleotide probe, indicating that the newly identified elements also carried the PBS complementary to the initiator tRNA.

Distribution and activity of retrotransposons in the rice genome

The copy number and the distribution of the elements in the rice genome were examined by the Southern hybridization analysis[16]. As shown in Plate 2, the copy number of Tos1, Tos2 and Tos3 was about 30. An indication in regard to the time when the elements were transposed in connection with the time when each subspecies of japonica and indica was formed could also be observed in the same hybridization. The pattern of the hybridizing bands for each element was similar among the different cultivars of japonica rice. However, the patterns of hybridization for Tos1 and Tos3 were different between the japonica and indica rice cultivars. These results indicated that the distribution of Tos1 and Tos3 were different between the japonica and indica genomes. This was further confirmed by the amplification of the target sequence of the elements

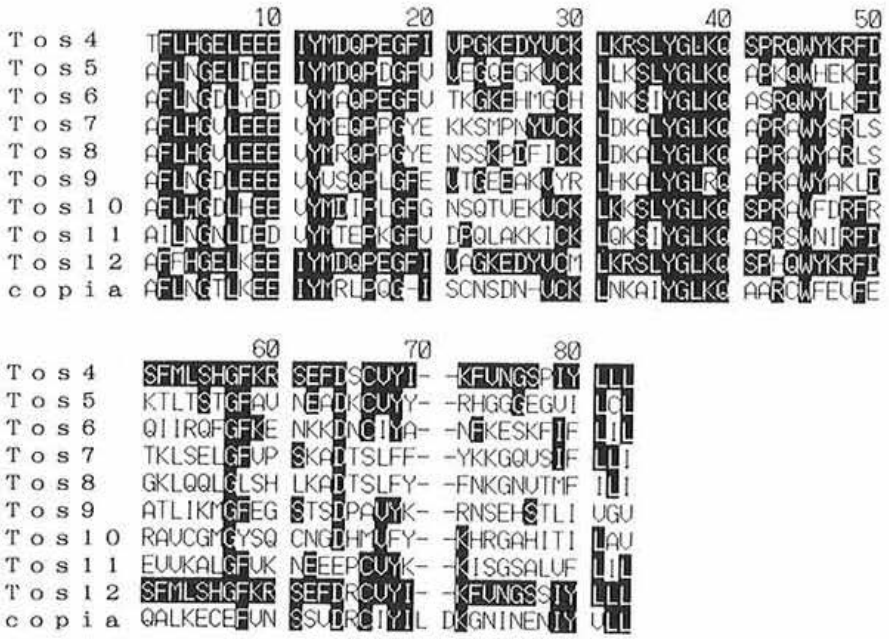

Fig. 3. Sequences corresponding to the reverse transcriptase domain

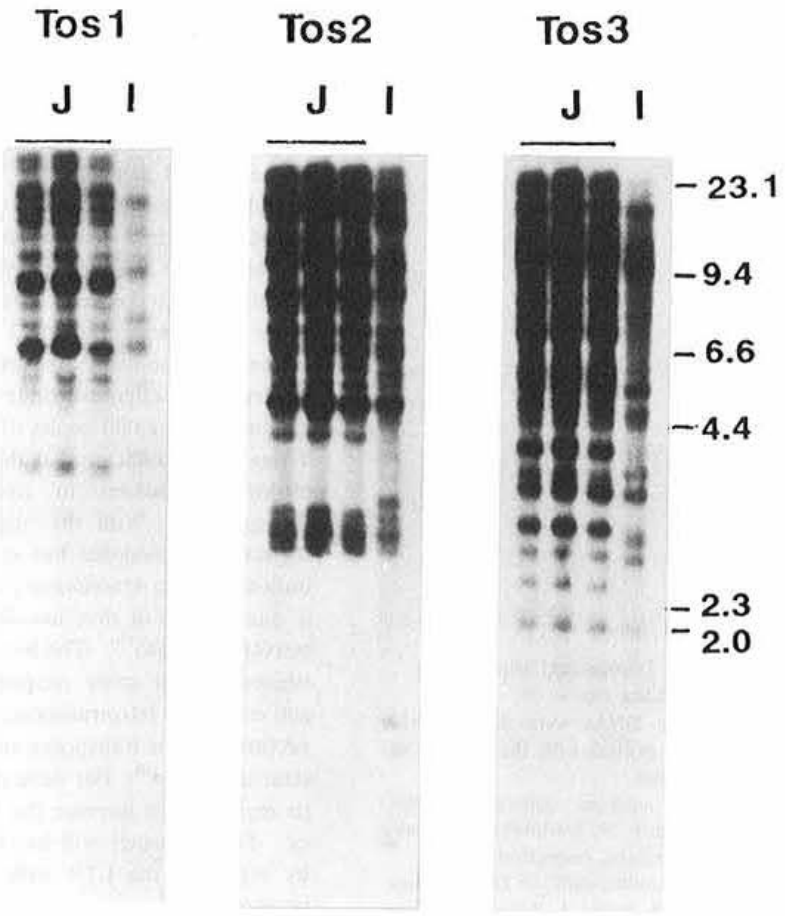

Plate 2. Distribution of sequences related to Tos1, Tos2, and Tos3 in japonica and indica rice

Three japonica cultivars (indicated by J: Norin 28, Koshihikari and Nipponbare from left to right) and one indica cultivar (indicated by I: Dee-geo-woo-gen) were analyzed.

The genomic DNAs were digested with *Eco*RI and probed with Tos1-, Tos2- and Tos3-specific probes[15].

by PCR. These results suggested that Tos1 and Tos3 transpose during or after the indica-japonica differentiation. The hybridization with the cloned sequences of Tos4-Tos12 showed that the distribution of all these elements was different between the japonica and indica genomes. The copy number and distribution of some elements were different even among the closely related cultivars. One example of the hybridization is shown in Plate 3. These results suggest that some retrotransposons be active in rice.

The presence of the Tos-related sequences in wild rice species and other unrelated species was examined by the Southern blotting analysis[16]. The Tos1, 2 and 3 were widely distributed in wild rice species but not in *Nicotiana tabacum* and *Zea mays*.

Discussion

As mentioned above, Ac-like elements (RAc) of rice were isolated, the evidence obtained suggested that these elements be active in the rice genome. However, the frequency of transposition may not be high enough, because no unstable mutations were

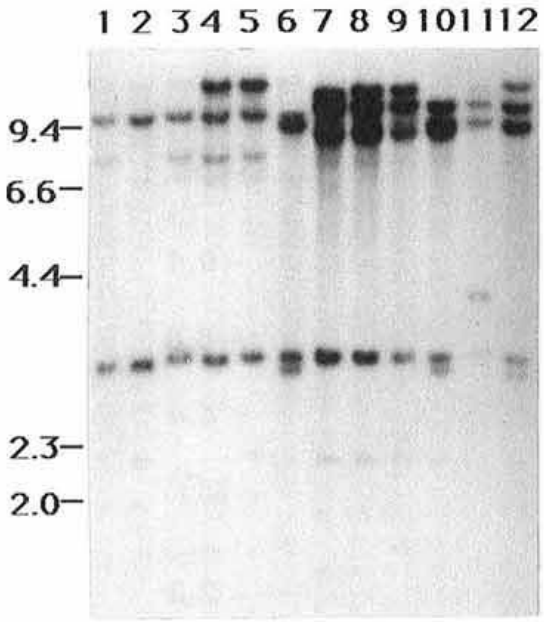

Plate 3. Distribution of Tos8-related sequences in japonica and indica rice
The genomic DNAs were digested with *Hin*dIII and probed with the labelled sequence of Tos8.
Lanes 1–6: japonica cultivars Aikoku, Norin 10, Norin 29, Koshihikari, Fujisaka 5 and Nipponbare, respectively.
Lanes 7–12: indica cultivars Dee-geo-woo-gen, Taichung Native 1, Tsai-yuan-chung, IR 8, Culture 340, and Peku, respectively.

reported in the cultivars which presented the transposition of RAc. In order to develop the tagging system, the transposition frequency should be increased. In maize, cryptic Ac elements existing in the genome can be activated by irradiation or tissue culture[20,23]. The same treatment may be effective in increasing the transposition frequency of RAc. Another possibility is to use the mutable lines of rice. Two mutable lines of rice are reported[28,29], in which some mutations reverted at a high frequency and the revertants produced various mutants. It is likely that the transposable elements such as RAc are involved in these mutable traits. The experiments to examine this possibility are now in progress.

The second type of transposable elements, i.e. retrotransposons, was isolated by using two novel methods. The first method using the oligonucleotide probe was very effective and it would probably be applicable to cloning any types of retrotransposons. The second method using PCR was quite simple and very effective. By using the latter method, some retrotransposons have recently been identified from 30 plant species including moss, gymnosperms and angiosperms[15]. However, the PCR method is applicable only to the known types of retrotransposons, such as the copia type described in this paper. For example, no sequences are amplified from the Tos3 type retrotransposons. By using those two methods, 12 families of retrotransposons were isolated. The screening of the genomic library by the oligonucleotide suggested that the rice genome carry 1,000 copies of the retrotransposons. These results indicate that the retrotransposons are major transposons in rice as they were in *Drosophila*[2]. With the oligonucleotide screening method, the evidence has recently been obtained, indicating that *Arabidopsis thaliana*, whose genome is one-seventh of rice, has about 200 copies of the retrotransposons[15]. The Southern blotting analysis suggested that some retrotransposons of rice be active. These retrotransposons will be used as gene vectors and for transposon tagging, as well demonstrated in yeast[4]. For these purposes, it is essential to regulate and increase the transposition frequency. The frequency will be regulated and increased by replacing the LTR with regulated and strong promoters[4].

Tissue-culture induced mutation, termed somaclonal variation, has been reported in many plant species[24] including rice. Somaclonal variation has been extensively studied as a source of variability for plant breeding. However, the mechanisms for somaclonal variation have not been well understood. A number of possible mechanisms have been proposed[24]. Because the activation of transposable elements has been demonstrated in tissue culture of maize[23], it seems likely that the transposable elements are, at least in part, responsible for somaclonal variation. Because cloned sequences of transposable elements of rice are now available, it is possible to examine whether transposable elements are responsible for somaclonal variation in rice.

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